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14. ABSTRACT
Patients undergoing chemotherapy can experience a decline in cognitive abilities. While well described from a clinical perspective, little is known of the neurological substrate for this difficulty, commonly known as 'chemo brain.' We hypothesize that the cognitive difficulties experienced by patients undergoing chemotherapy are the result of impaired neurogenesis, especially in the hippocampus. We further hypothesize that agents that do not cross the blood-brain barrier will not show reduced rates of neurogenesis, in contrast to agents that readily cross into the central nervous system (CNS). Our objective is to compare the effect of drugs that enter the CNS (Cytoxan and 5-FU) with agents that do not (Adriamycin and Taxol) with respect to their ability to impair the birthing of new neurons in the hippocampus of adult mice. By testing whether chemotherapeutic agents that enter the CNS can reduce neurogenesis, we hope to develop an animal model of 'chemo brain' that will allow further studies. Furthermore, if we can show that inhibition of neurogenesis is a correlate of behavioral decline after chemotherapy, we will have provided evidence that modification of chemotherapeutic regimens – specifically, using strategies to prevent CNS entry of drugs – would be of great importance in improving the quality of life in cancer patients.

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INTRODUCTION:

The purpose of the work was to test the hypothesis that chemotherapeutic agents can cause a reduction in neuronal birthing in the hippocampus. The rationale for this proposition is that the hippocampus is a crucial structure for memory function; as such, any disruption to its normal functioning – including disruption of the normal process of neuronal birthing – could lessen memory function. Such a process could underlie the condition popularly known as "chemo brain," in which persons who have received chemotherapy have reduced cognitive skills. To test this hypothesis, we chose chemotherapeutic agents of two groups, those that cross the blood-brain barrier (cyclophosphamide, 5-FU) and those that do not (doxorubicin, paclitaxel); we predict, based on our hypothesis, that those that readily enter the brain would reduce neuronal birthing, whereas there would be no effect on birthing by those agents that do not enter the brain. *A limitation to this series of controls is that detailed data regarding passage through the blood-brain barrier are not available.* Mice were to be treated with these agents, and bromo-deoxy-uridine (BrdU) would be injected, thereby labeling newly birthed cells. By use of immunohistochemical techniques, we sought to determine the number of birthed neurons and their destiny – for example, differentiation into neurons or glia.

REPORT:

Extenuating Circumstances:
The progress has been slow due to unforeseen circumstances. The original technician left unexpectedly (to enter an MD/PhD program), and it proved difficult to find another person with the requisite skill set. Training took several months. In addition, we were using the imaging facilities of a collaborator, who unexpectedly left this institution, leaving us temporarily without adequate microscopy support. The original pilot experiment, designed to test the safety and efficacy of the chosen chemotherapeutic regimens was finally completed, but with results that could not be interpreted with certainty (below). A second pilot was delayed until the proper technical support was in place. The second pilot and a definitive trial are now completed.

We have asked for and received a second twelve-month extension to continue this work.

Report:

1) We have completed three experiments.

<u>Experiment #1: 4 mice per treatment group; 12 controls</u>

- Doses of drug administered on days 1, 4 and 7:
  - cyclophosphamide 50 mg/kg
  - 5-FU 100 mg/kg or 60 mg/kg – reduced because of ill health or death (75%) in higher dosage group
  - Doxorubicin 5 mg/kg
  - (no paclitaxel)
- BrdU administered on day 8, 4 injections q2hr 50 mg/kg
- Animals sacrificed 28 days after BrdU (to allow maturation)

Experiment #2: 5 mice per group, 10 controls

- Doses of drug administered on days 1, 4 and 7:
  - 5-FU 60 mg/kg
  - Paclitaxel 5 mg/kg
- BrdU administered as 4 injections q2hr on days 8-11 50 mg/kg
- Animals sacrificed 14 days after last BrdU injection

Experiment #3: 6 mice per group, 8 controls

- Doses of drug administered on days 1, 4 and 7:
  - 5-FU 60 mg/kg
  - Paclitaxel 5 mg/kg
  - Cyclophosphamide 50 mg/kg
  - Doxorubicin 5 mg/kg
- BrdU administered as 4 injections q2hr on day 8
- Animals sacrificed on day 9

We thus have experiments that allow us to assess maturation, with neuronal birthing in all cases occurring just after chemotherapy. Full maturation of birthed cells (experiment 3), partial maturation (experiment 2) and an immediate assessment of the birthing rate without maturation (experiment 1).

For all experiments, tissue was fixed *in vivo* and processed for chromagen staining with biotin-avidin. Chromagen staining was performed on every third hippocampal slice.

2) Results:

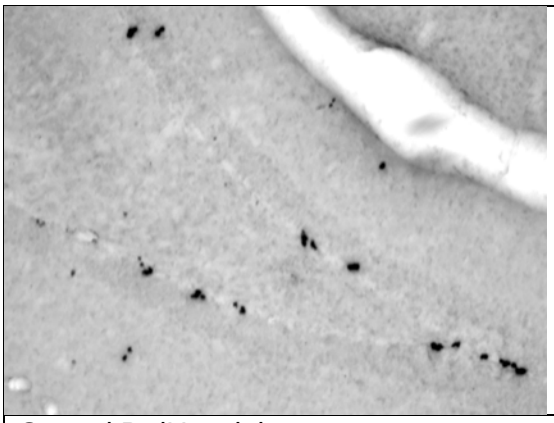
While this experiment suggested that the chemotherapeutic regimens chosen were relatively safe – in other words, that toxicity would not be a major confound – we still noted that weights declined slightly in the doxorubin- and 5-FU-treated mice, by 10-15% and 5%, respectively.

Experiments 1 and 2: After the definitive experiment (#3), it became evident that the results of these two experiments could not be used because the sectioning technique was insufficient to provide consistent cuts through the hippocampus. We have stored one hemisphere intact, however, and will go back and section the second hippocampus to provide data on maturation of newly birthed cells. One limitation, especially for the first experiment, is that exact numbers of cells may be difficult to determine: while a 28-day period after BrdU allows time for differentiation of newly-birthed cells to neuronal or glial types, it also reduces cell counts due to cell death.

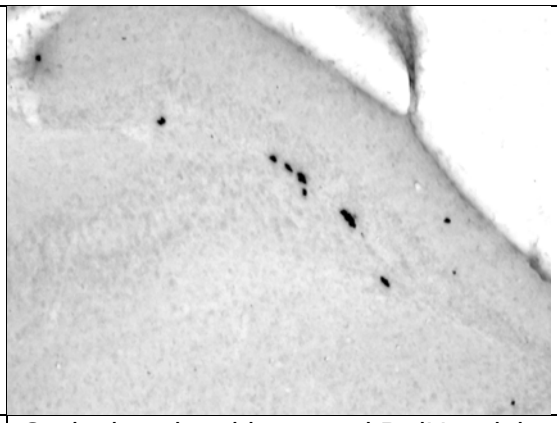
Experiment 3:

This experiment measures the effect of chemotherapeutic agents on cell birthing in the hippocampus. Sacrifice of animals the day after BrdU administration allows for comparison to

Experiment 1, which followed the same drug and BrdU schedules, but allowed for maturation of cells. In the Figure, a typical control and treated hippocampal slice are shown.



Control BrdU staining



Cyclophosphamide-treated BrdU staining

Condition	**Cells / Slice**	**Cells / Hippocampus (% change from Control)**
	Mean ± SEM	
Controls	35 ± 1	2103 ± 56
5-FU	30 ± 1	1777 ± 57 (-16*)
Cyclophosphamide	24 ± 0.8	1462 ± 51 (-30**)
Paclitaxel	27 ± 1	1632 ± 58 (-22**)
Doxorubicin	23 ± 1	1360 ± 68 (-35**)

* $p < 0.002$
** $p < 0.001$

3) Interpretation:

This experiment confirms our overall hypothesis, that chemotherapeutic agents may reduce cell birthing in the hippocampus, and may thus underlie the condition known as ‘chemo brain.’

However, the design of the experiment was such that we included agents thought to cross the blood-brain barrier (5-FU, cyclophosphamide) and those thought not to cross (paclitaxel and

doxorubicin). The experiment was designed so that there was an internal control consisting of agents that ought not to affect neuronal birthing. Data on chemotherapeutic agents crossing the blood-brain barrier are sparse, however, and we made assumptions based on human experience, as mice data were not definitive. Our result thus suggests either 1) that agents that penetrate the CNS poorly in humans may still penetrate sufficiently to affect hippocampal cell birthing; or 2) that the agents that penetrate the CNS poorly may have secondarily disrupted the blood-brain barrier, for example by affecting overall health. In this context it is worth noting that the drugs that affected health (as measured by weight) most, 5-FU and doxorubicin, had disparate effects on birthing, being the most and least suppressive.

Having worked out the technical details, and having shown a robust significant reduction of cell birthing resulting from all four chemotherapeutic regimens, a number of questions still remain unanswered. Experiment 3 shows that cell birthing is reduced, but does not speak to the eventual fate of those cells. To address this, we will section and stain tissue from Experiments 1 and 2, so as to determine the cell fates. If our previous results are a reliable guide (references), we expect that cell maturation will have begun by 2 weeks after labeling (Experiment 2) and that it will have nearly completed by 4 weeks (Experiment 4). Slices will be stained for BrdU and for the neuronal marker NeuN (and, if necessary, for the glial marker GFAP) and counted. The proportion of BrdU-positive cells that are neuronal, glial or neither will be tabulated and, for each chemotherapeutic regimen, compared to control. We expect to similar proportions of new cells evolving to neuronal or glial lineages, although it is possible that inhibition of cell birthing could skew the development toward a specific cell type.

To verify that mice treated with chemotherapeutic agents may serve as a model for chemo brain, behavioral measures should be correlated with cell counts. We hypothesize that animals with lower cell / neuronal counts will be poorer learners. At present, we are consulting with colleagues skilled in animal learning paradigms who will help plan appropriate experiments.

REPORTABLE OUTCOMES:

No publications or grant applications to date.

CONCLUSIONS:

Our results to date are sufficient to address our hypothesis, that chemotherapeutic agents may cause “chemo brain” because of an effect on cell birthing in the hippocampus. We have yet to complete the experiments that will determine newly-birthed cell differentiation and are exploring the possibility of behavioral experiments.

The hypothesis remains testable and important: if we can show an effect on neuronal birthing, a number of potential discoveries follow. Strategies that reduce penetration of chemotherapeutic agents into the CNS may allow adequate systemic treatment, but without the psychological-behavioral consequences.

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